
A NEW EXPLANATION FOR THE EVOLUTION OF POLLINIA AND LOSS OF CARPEL FUSION IN *ASCLEPIAS* AND THE APOCYNACEAE S.L.¹

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ABSTRACT

The demonstration of postzygotic self-incompatibility in *Asclepias* L. prompted us to reconsider explanations for several unusual features of the Apocynaceae. With such late-acting self-incompatibility, mixed loads of self- and cross-pollen can be extremely wasteful by causing abortion of whole fruits and consequent wastage of both cross-pollen and cross-fertilized ovules. We contend that the evolution of pollinia and loss of carpel fusion in certain Apocynaceae represent adaptations to prevent or compensate for these negative effects of mixed pollen loads. Trends in the agglutination of pollen can be seen in the tetrads of *Apocynum* L., which are transported on sticky band-like translators; the masses of tetrads of Periplocoideae, which are deposited onto the sticky scoop portion of a spoon-like translator; the pollinia of Secamonoideae, which have a clip-like translator but no outer covering; and the pollinia of Asclepiadoideae, which are completely enclosed by a waxy outer covering. In many genera of the Rauvolfioideae, the ovary is syncarpous, but in most Apocynoideae and all Periplocoideae and Secamonoideae, the gynoceum consists of two carpels that are free in the ovule-bearing region but fused in the upper region to produce a compitum, which enables pollen placed on one stigmatic area to supply pollen tubes to both ovaries. In at least some Asclepiadoideae, however, a compitum is lacking, although postgenital fusion between the carpel apices still takes place. Parallel trends in pollen delivery and receipt also occur in taxa from other angiosperm families in which late-acting self-incompatibility systems have been implicated.

Key words: Apocynaceae, Apocynoideae, Asclepiadoideae, *Asclepias*, carpel fusion, compitum, late-acting self-incompatibility, Periplocoideae, pollinia, Secamonoideae.

In a letter to Asa Gray in 1857, Charles Darwin wrote:

“The facts which kept me longest scientifically orthodox are those of adaptation—the pollen-masses in *Asclepias*. . . To talk of climate or Lamarckian habit producing such adaptation to other organic beings is futile. This difficulty, I believe I have surmounted.” (Burkhardt & Smith, 1990: 445)

Given the state of knowledge of genetics in 1857, Darwin certainly can be forgiven for his inability to explain the evolution of milkweed pollinia. Moreover, it is our contention that the explanation lies not in the arena of pollinator–plant coevolution, as Darwin imagined, but rather in the genetics of self-incompatibility. Our work has shown that poke milkweed (*Asclepias exaltata* L.) possesses a postzygotic self-incompatibility system (Lipow & Wyatt, 2000). Such late-acting self-incompatibility is extremely inefficient, often resulting in wasted cross-pollen and cross-

fertilized ovules when mixed loads of self- and cross-pollen reach stigmas. This has led to selection for traits that decrease the likelihood of this costly mixing occurring.

These traits include androecial and gynoecial features that affect both pollen delivery and receipt. In the subfamilies Apocynoideae, Periplocoideae, Secamonoideae, and Asclepiadoideae (hereafter, APSA clade), there are clear trends toward agglutination of pollen, culminating in complex pollinia, specialized structures that transport large numbers of pollen grains as discrete units, such that the likelihood of mixing of pollen from different donors is minimized. Pollinia are rare, occurring among dicots only in certain derived Apocynaceae, although some other families produce pollen grains that are transported in multiples. Among monocots, analogous (but certainly not homologous) pollinia occur in the Orchidaceae. In the Apocynaceae, there are also evolutionary trends toward increased separation of the

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ovaries and the gynoecial transmitting tissue leading to secondary apocarpy, a trend exactly opposite that found in nearly all other groups of angiosperms (Endress, 1982, 1990). In many Rauvolfioideae, the ovary is syncarpous, but in most Apocynoideae and all Periplocoideae and Secamonoideae, the gynoecium consists of two carpels that are free below and united above in the styler region. In *Asclepias* L. of the Asclepiadoideae, these apically fused carpels lack a compitum, a shared transmitting tract that distributes pollen tubes to all carpels regardless of the stigma on which pollen is placed (Sage et al., 1990). Therefore, pollen from a given pollinium can reach only one of the two ovaries, again minimizing the likelihood that an ovary will receive pollen from multiple donors.

Here we present a new explanation for the evolution of pollinia and loss of carpel fusion in milkweeds. We then briefly review recent phylogenetic studies of the Apocynaceae s.l. and relate these to evolutionary trends in pollen agglutination and loss of carpel fusion. We also consider earlier explanations for the evolution of pollinia and secondary apocarpy. Finally, we evaluate parallel evolutionary trends in other families of flowering plants and the ability of our new hypothesis to explain them.

A NEW EXPLANATION

It seems likely that postzygotic self-incompatibility is widespread in *Asclepias*. At least seven species of *Asclepias* are predominantly self-sterile (reviewed by Wyatt & Broyles, 1994; Wyatt et al., 1996, 1998). Although self-pollen tubes grow normally and effect fertilization, fruits are rarely (or never) produced following self-pollinations (Sparrow & Pearson, 1948; Kephart, 1981; Sage & Williams, 1993; Wyatt et al., 1996). Instead, the selfed zygotes do not undergo mitosis, the endosperm divides only a few times, and the selfed ovules consistently abort. Full-sib diallels performed on *A. exaltata* have demonstrated that self-sterility is due to a single gene, apparently with many alleles segregating in local populations (Lipow & Wyatt, 2000). Crosses are incompatible whenever an allele at the S-locus of the diploid pistil matches an allele of the diploid pollen.

Crossing and microscopic studies have identified species in several other genera within the APSA clade that are also self-sterile but show self-pollen tubes growing normally and entering ovules: *Apocynum cannabinum* L. (Apocynoideae), *Periploca aphylla* Decne. (Periplocoideae), and *Gonolobus suberosus* (L.) R. Br. (Asclepiadoideae) (Lipow, 1998; Lipow & Wyatt, 1998, 1999). The genetics that underlie recognition and rejection of self-pollen in these and other genera of the APSA clade are likely the same as

in *A. exaltata*, as self-incompatibility systems are typically conserved among closely related taxa (de Nettancourt, 1977). Certainly, the behavior of all these taxa with respect to experimental crosses and ovule penetration is remarkably consistent.

We are not aware of any studies that have examined in detail the nature of self-incompatibility in the Rauvolfioideae (the basal subfamily of Apocynaceae), especially the possibility that it might be late-acting. Such studies could be revealing, however, because in species from several families of Gentianales, incompatible pollen tubes have been observed to arrest in the ovules (Bawa, 1974, 1979; Bawa et al., 1985). The five families currently recognized in the Gentianales fall into two main clades. One is composed solely of the Rubiaceae, whereas the other includes the remaining four families in the following relationship: Apocynaceae are sister to Gelsemiaceae, which together are sister to Loganiaceae s. str., and all three families together are sister to Gentianaceae (Backlund et al., 2000).

We contend that the evolution of postzygotic self-incompatibility created conditions in which compatible cross-pollen was wasted whenever it occurred in mixture with incompatible pollen, because such mixed pollen loads would likely cause the entire fruit to abort. This is because crosses between plants that share even a single self-incompatibility allele are incompatible (e.g., $S_1S_3 \times S_1S_4$). Such diploid control over the compatibility phenotype of the pollen is surprising. Rejection of pollen occurs after penetration of ovules by pollen tubes, but pollen tubes express the haploid, not diploid, male genotype. Thus, in these crosses, one half of the ovules should receive compatible pollen and one half should receive incompatible pollen. Two explanations can account for the observed failure of any seeds to mature from such crosses: (1) recognition occurs much earlier than rejection (hence, the “decision” to abort the fruit is made before fertilization occurs), or (2) fruit maturation requires that more than one half of the ovules within an ovary receive compatible pollen (which seems to be the case in *Asclepias*, as such crosses invariably abort, with fruit-set occurring only from ovaries with a full complement of compatibly fertilized seeds). In either case, a mixed pollen load containing half compatible and half incompatible pollen is unlikely to mature any seeds. This wastes not only incompatible pollen, but also compatible pollen and fertilized ovules. This maladaptive condition may have been the stimulus for the evolution of pollinia and loss of the compitum.

EVOLUTIONARY INNOVATION AND DIVERSIFICATION IN FLORAL MORPHOLOGY

Within the Apocynaceae, it is possible to discern various levels of evolutionary complexity relating to

the formation of pollinia and loss of carpel fusion, as well as several other unique morphological features (Struwe et al., 1994; Liede, 1996; Sennblad & Bremer, 1996; Endress & Bruyns, 2000; Livshultz et al., 2007). Five major groups are often recognized in the Apocynaceae s.l. and assigned subfamilial rank: the Rauvolfioideae and Apocynoideae of the Apocynaceae s. str. and the Periplocoideae, Secamonoideae, and Asclepiadoideae of the Asclepiadaceae (Rosatti, 1989; Leeuwenberg, 1994; Liede & Albers, 1994). The Rauvolfioideae have often been interpreted as a paraphyletic group, from which the other subfamilies have radiated (Schumann, 1895; Pichon, 1948; Fallen, 1986; Sennblad & Bremer, 1996).

Here we focus on the APSA clade. These four subfamilies form a strongly supported monophyletic group characterized by anthers that are adnate to the style-head forming a gynostegium, dextrorse corolla lobe aestivation, an apocarpous gynoecium, and dehiscent follicles containing small seeds with a coma on the micropylar end (Livshultz et al., 2007). Systematic relationships within this group have been controversial. Historically, the Periplocoideae have usually been classified in the Asclepiadaceae (Brown, 1810; Bentham, 1876; Schumann, 1895), but morphological and phylogenetic studies conducted over the past 10 years have suggested, albeit with poor resolution, that the traditional Asclepiadaceae may be biphyetic, with the Periplocoideae and the Secamonoideae–Asclepiadoideae each having a closer relationship to a different group of taxa within the Apocynoideae than they do to each other (Kunze, 1996; Sennblad & Bremer, 1996; Potgieter & Albert, 2001). The close relationship between Secamonoideae and Asclepiadoideae has long been accepted based on morphology alone (Safwat, 1962; Kunze, 1993; Liede, 1996; Fishbein, 2001), and molecular results have confirmed they are sister groups that together form a monophyletic clade (Sennblad & Bremer, 1996). In the broad-based phylogenetic study by Livshultz et al. (2007), resolution within Apocynoideae is much improved, illuminating relationships within the APSA clade, including strong support that the closest relative of Secamonoideae–Asclepiadoideae is a clade of three closely related genera of Apocynoideae, rather than Periplocoideae, and thus confirming the polyphyly of the traditional Asclepiadaceae, a conclusion reached independently by Lahaye et al. (2007).

Evolution of pollinia. The highly derived pollinia of *Asclepias* represent the extreme of an evolutionary trend in the Apocynaceae toward increased agglutination of pollen (Endress, 1994; Endress & Bruyns, 2000). In most genera of the Rauvolfioideae and Apocynoideae, pollen is released as single grains

from anthers with two thecae, each of which contains two pollen sacs. In the Apocynoideae, the anthers become postgenitally united with the style-head in various ways. In all subfamilies, the style-head secretes sticky substances that serve as glue for pollen transport (Endress, 1994). Among the Apocynoideae, *Apocynum* L. (Apocyneae) has pollen that is dispersed as tetrads and simple band-like translators that are formed through desiccation of the style-head secretions; band-like translators also occur in *Forsteronia* G. Mey. (Mesechiteae) (Schick, 1982a; Nilsson et al., 1993; Fig. 1A, B). This aggregated transport and mass transfer may reduce the mixing of self- and cross-pollen on the stigmatic surface.

In the Periplocoideae, we see more dramatic evidence of a pollen delivery mechanism that minimizes pollen mixing. Pollen is deposited onto an elaborate translator, which is typically differentiated into a spoon-like pollen receptacle and a stalk with a sticky base (Schick, 1982b; Kunze, 1993; Venter & Verhoeven, 1997; Fig. 1C, D). During pollination, the sticky base of the stalk becomes attached to an insect. When the insect leaves the flower, it pulls out the spoon of pollen, which subsequently may be brought into contact with the stigmatic surface of the style-head when the insect visits another flower (Schick, 1982b). In *Periploca aphylla*, each spoon contains ca. 125 times more pollen grains than there are ovules within an ovary (Wyatt et al., 2000). Thus, a single pollination could deliver more than enough pollen grains to fertilize all of the ovules within an ovary. Pollen is usually shed in tetrads, but in seven of the 33 currently recognized genera, the tetrads of each pollen sac are loosely coherent into pollinia, which lack a sporopollenin outer wall and are not considered to be homologous to those in the Asclepiadoideae–Secamonoideae lineage (Fishbein, 2001; Ionta & Judd, 2007). The pollinia from two pollen sacs of two adjacent anthers are shed onto the adhesive-lined spoon of the translator. Verhoeven and Venter (2001) concluded that pollinia have evolved independently at least twice, and results of a recent molecular analysis of the subfamily indicate that there were probably three to four independent origins of pollinia in the Periplocoideae alone (Ionta & Judd, 2007).

Simultaneous transport of numerous pollen grains in pollinia also evolved independently in the Secamonoideae–Asclepiadoideae lineage. The pollinia of Secamonoideae are superficially similar to those found in Periplocoideae, in being composed of coherent tetrads and lacking an outer sporopollenin wall. Differences in details of the tetrad wall structure (Verhoeven & Venter, 2001; Verhoeven et al., 2003), however, convinced Ionta and Judd (2007) that the

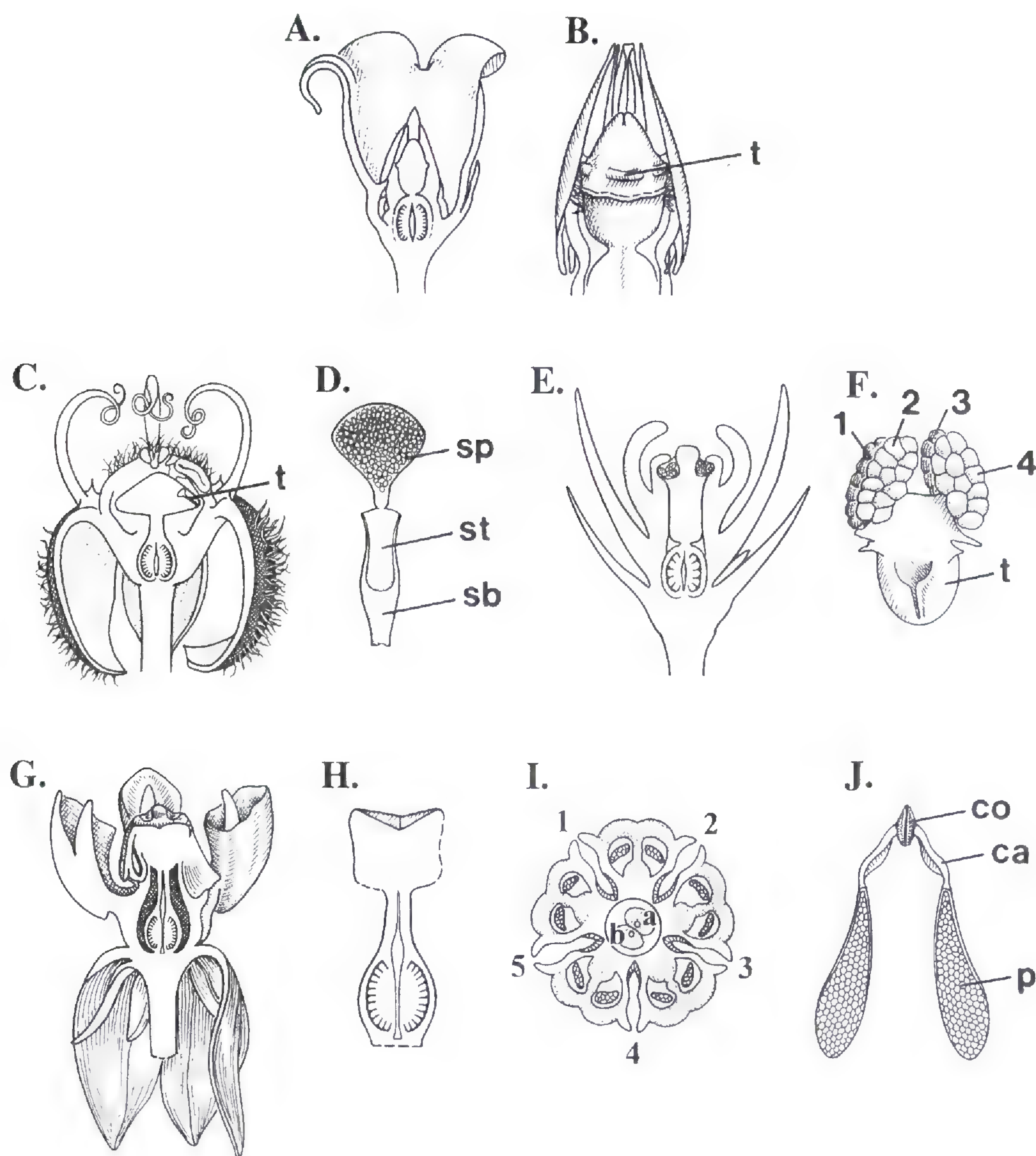


Figure 1. Flower morphology of plants selected to represent some major morphological transformations in the evolutionary history of the Apocynaceae s.l. A–B. *Apocynum* (Apocynoideae). —A. Longitudinal section, showing the two carpels that are free in the ovule-bearing region but fused above to form a style-head with a compitum. —B. Surface view of a flower from which two anthers have been removed to reveal the band-like translators (t) on the style-head. C–D. *Periploca* (Periplocoideae). —C. Longitudinal section, showing the two carpels that are free in the ovule-bearing region but fused above to form a style-head with a compitum. The translator (t) rests on the surface of the style-head. —D. A translator consisting of a stalk (st) with a sticky base (sb) and a spoon (sp) filled with pollen tetrads. E–F. *Secamone* (Secamonoideae). —E. Longitudinal section, showing the two carpels that are free in the ovule-bearing region but fused above to form a style-head with a compitum. —F. A pollinium consisting of four sacs of pollen (1–4; note that the two shaded sacs lie in a plane below the two others) and a translator (t). G–J. *Asclepias* (Asclepiadoideae). —G. Longitudinal section, showing the location of the style-head relative to the style and ovary. —H. Longitudinal section through the two carpels, which are joined only at their apices; the carpels lack a compitum. —I. Transverse section, showing the location of the stigmatic chambers relative to the styles. Chambers 1, 2, and 3 transmit pollen tubes to style (a), whereas chambers 4 and 5 transmit pollen tubes to style (b). —J. A pollinarium consisting of two sacs of pollen or pollinia (p) joined by a translator, composed of a corpusculum (co) and two caudicles (ca).

pollinia of these two subfamilies are not homologous. As in Periplocoideae, each pollinium in Secamonoideae contains four pollinia, resulting from the four pollen sacs in two thecae. There is no spoon-shaped translator; instead, a clip-like translator (often called a corpusculum) joins two pollen sacs from each of two adjacent anthers (Fig. 1E, F).

The pollinia of the Asclepiadoideae (except *Fockea* Endl.; Verhoeven et al., 2003) are structurally different from those of the Periplocoideae and Secamonoideae. Pollinia in Asclepiadoideae are typically interpreted as being composed of single grains (Verhoeven & Venter, 2001; Verhoeven et al., 2003; but see Livshultz et al., 2007) and are

surrounded by an outer wall formed of sporopollenin (Dannenbaum & Schill, 1991; Verhoeven & Venter, 2001). Furthermore, only one of the two pollen sacs per theca is fertile, so that each anther produces only two pollinia. Pairs of pollinia from two adjacent anthers are joined to a corpusculum by two flexible arms (caudicles) to form a pollinarium (Kunze, 1996; Liede, 1996; Fig. 1G–J). Pollination is accomplished when the corpusculum becomes clipped onto an insect's appendage. As the insect frees itself, it removes the entire pollinarium from the flower. Subsequently, a pollinium may be inserted into a stigmatic chamber (some Asclepiadeae) or wedged against a guide-rail (most other taxa), completing pollination. Again, each pollinium contains more than enough pollen grains to fertilize all of the ovules within an ovary (Wyatt et al., 2000), and at least for species studied thus far in *Asclepias*, a single father typically sires all of the seeds within a fruit (Broyles & Wyatt, 1990; Gold & Shore, 1995).

The presence of pollinia ensures that all of the pollen delivered to the stigmatic surface during a pollination event will be either compatible or incompatible. Based on experimental studies of *Asclepias exaltata*, incompatible pollen can usurp ovules and prevent them from developing, resulting in an aborted follicle (Broyles & Wyatt, 1993). Compatible pollinations, however, will result in follicles that contain a full complement of cross-pollinated seeds (cf. Broyles & Wyatt, 1990). When multiple pollinia were inserted to direct compatible and incompatible pollen tubes to the same ovary, fruit-set was reduced as much as 81% (Broyles & Wyatt, 1993).

Evolutionary loss of fused carpels. The extent of fusion of the carpels has traditionally been used to delimit infrafamilial groups within the Apocynaceae (Endlicher, 1841; Bentham, 1876; Schumann, 1895; Ly, 1986). Use of this character, however, has proved troublesome, as both congenital and postgenital syncarpy occur in the family (Fallen, 1985). Moreover, Sennblad and Bremer (1996) have shown that many tribes within the Rauvolfioideae defined on the basis of carpel number and fusion are paraphyletic. Phylogenetic reconstructions support the view that the most basal taxa in the Apocynaceae are apocarpous (Potgieter & Albert, 2001) and that congenital syncarpy has arisen independently several times within the Rauvolfioideae (Simões et al., 2007). Syncarpous taxa are also known from the other four subfamilies, but in all of these studied thus far, it appears that fusion is postgenital (M. E. Endress, pers. comm.).

The gynoecium of Apocynoideae typically consists of two carpels that are entirely free in the ovule-bearing region, but that undergo postgenital fusion of

their upper parts to give rise to a complex new organ known as the style-head (Fig. 1A, B). This postgenital fusion of the upper part of the style produces a functional compitum (Walker, 1978; Endress, 1982; Lipow & Wyatt, 1999). Based on very limited sampling of taxa, Periplocoideae and Secamonoideae appear to retain this basic gynoecial plan (Kunze, 1991; S. Lipow, unpublished data). In these subfamilies, the transmitting tissue shares a common beginning in the style-head so that a single inserted pollinium can supply pollen tubes to both ovaries (Fig. 1C, E).

In at least some Asclepiadoideae, however, there is no functional compitum, even though postgenital fusion between the carpel apices takes place (Fig. 1G, H). This has been demonstrated in *Leptadenia* R. Br. of the Ceropegieae (Kunze, 1991) and in the following genera of the Asclepiadeae: *Asclepias* (Sparrow & Pearson, 1948; Sage et al., 1990), *Gonolobus* Michx. (Lipow & Wyatt, 1998), *Microlooma* R. Br. (P. Bruyns, cited in Kunze, 1991), *Sarcostemma* R. Br. (Kunze & Liede, 1991), and *Vincetoxicum* Wolf (Kunze, 1991). In these taxa, it is also rare for both ovaries from one flower to mature (i.e., twin fruits), whereas twins are otherwise the rule in the APSA clade. In the absence of a compitum, production of twin fruits requires at least two insertions of compatible pollen into appropriate stigmatic regions, because each of the five separate stigmatic areas communicates with only one ovary (Fig. 1I). Three of the five stigmatic regions transmit pollen tubes to one ovary, and the other two stigmatic regions transmit pollen tubes to the second ovary (Sage et al., 1990). Apparently, multiple depositions of compatible pollinia on stigmatic areas leading to separate ovaries are rare, as twin fruits are uncommon in *Asclepias* (Wyatt & Broyles, 1994) and, presumably, in other taxa that lack a functional compitum.

We contend that some of the curious evolutionary trends within the Apocynaceae involving loss of carpel fusion can be explained as ways to reduce the wasteful effects of an inefficient postzygotic self-incompatibility system. Endress (1982) speculated that gynoecia with free carpels might have an advantage over syncarpous ones because ovaries in which few or no ovules have been fertilized will not consume limited resources by participating in fruit maturation. Indeed, postzygotic self-incompatibility greatly increases the likelihood of ovaries that contain only a few compatibly fertilized ovules. With syncarpy, the majority of ovaries might receive both self- and cross-pollen tubes and, in these, self-pollen will usurp some proportion of the available ovules and prevent them from developing into viable seeds. Separating the ovaries therefore provides plants with

a means to mature only those ovaries that contain a high proportion of compatibly fertilized ovules. Thus, the evolution of postzygotic self-incompatibility may have created an unusual circumstance in which apocarpny had a clear selective advantage. Noting that “syncarpny has evolved repeatedly in the angiosperms and must be highly adaptive,” Armbruster et al. (2002: 657) concluded that the paucity of reversals from syncarpny to apocarpny suggests that conditions favoring apocarpny over syncarpny are indeed “uncommon,” as they found only two cases other than the partial reversal in the Apocynaceae.

An even stronger case can be made that loss of the compitum in *Asclepias* and at least a few other genera of the Asclepiadoideae represents a means of avoiding some of the most wasteful consequences of late-acting self-incompatibility. A functional compitum distributes pollen tubes to all ovaries in a flower. In *Asclepias*, however, pollen from each stigmatic chamber can reach only one ovary (Fig. 1I), which reduces the likelihood of both self- and cross-pollen being delivered to any one ovary. The loss of the compitum therefore enables one ovary to mature a fruit, even if the other is aborted due to its having received a mixed load of pollen. Such mixed pollen loads appear to be quite common, with many milkweeds, especially those that spread vegetatively, displaying high rates of self-pollination (Woodson, 1930; Morse, 1982, 1994; Pleasants, 1991; Wyatt & Broyles, 1994; Gold & Shore, 1995).

EARLIER EXPLANATIONS

In his monograph of the genus *Asclepias* in North America, Woodson (1954: 22) noted the difficulty of explaining the evolution of pollinia and other morphological features of the milkweed flower and concluded that “entomophily, alone, is an unsatisfying generality.” Darwin too was at a loss to explain the origin of pollinia, although he continued to adhere to an adaptationist view (Burkhardt & Smith, 1990). The prevailing explanation, that pollinia confer a fitness advantage by virtue of increased efficiency of pollen delivery, was voiced earlier by one of the authors (Wyatt, 1976: 851): “This greater efficiency could represent a part of the advantage accruing to asclepiads with their pollen grains being transferred as a unit.” This conclusion was reached on the basis of data for *A. tuberosa* L., which showed that overall pollination efficiency was about 1.71% (i.e., for every 100 pollen grains produced, 1.71 were actually deposited on stigmas), a figure several times higher than values reported for species with loose pollen grains (Wyatt, 1976). The likelihood of selection for pollination efficiency being so strong and consistent

across so many derived taxa of the Apocynaceae, however, seems remote. This is especially true given the wide range of environments, including pollinator diversity, that various Apocynaceae experience. Moreover, it is striking that, despite having “the most elaborate, complicated flowers of all the dicots” (Endress, 1994: 302) and “extreme adaptation to insect pollination” (Safwat, 1962: 95), species of *Asclepias* have unexpectedly low fruit-set compared to most other groups of flowering plants (e.g., Wilbur, 1976; Wyatt & Broyles, 1994).

The pollination efficiency argument also fails to explain why pollinia should have evolved repeatedly as a solution to low efficiency of pollen transfer only in the derived Apocynaceae (among the dicots). Certainly, lineages in other plant families have faced this challenge, and many other solutions have been explored. In the Apocynaceae, selection for mechanisms promoting increased agglutination of pollen appears to be unusually strong, as pollinia probably evolved independently at least three times in the Periplocoideae (Ionta & Judd, 2007) and at least once in the Secamonoideae–Asclepiadoideae (Livshultz et al., 2007). Moreover, the pollination efficiency argument fails to account for the apparent association of pollen aggregation and mass transfer with late-acting self-incompatibility (although we admit that the occurrence of the latter is not well studied across the family).

Another shortcoming of the pollination efficiency argument is that it is based solely on data from *Asclepias*, a highly derived genus of the Asclepiadoideae (Rapini et al., 2003). As such, it may shed little light on the forces repeatedly driving the evolution of increased agglutination of pollen in other Apocynaceae. The pollen of *Periploca aphylla* (Periplocoideae), for example, is shed as tetrads and transported in a spoon-shaped translator (Fig. 1D). This species has a much higher pollen:ovule ratio of 128:1 than that of *Asclepias*, which is less than 2:1 for most species (Wyatt et al., 2000). In fact, the pollen:ovule ratio of *P. aphylla* approaches the range of some plants with loose pollen grains (Cruden, 1977). Thus, agglutination of pollen in *P. aphylla* does not appear to increase pollination efficiency as dramatically as expected, a finding consistent with Rosatti’s (1989: 454) speculation that “the mechanism of pollen transfer in the Periplocoideae is less effective than that in the Asclepiadoideae.”

We believe that the greater efficiency of pollen delivery is an epiphenomenon, a fortunate happenstance of the evolution of pollinia. The driving force behind the evolution of pollinia, however, was actually selection to prevent mixed pollen loads from reaching stigmas. This more general, genetic explanation seems

to accord well with the time of origin of the structures involved and of postzygotic self-incompatibility, although our knowledge of these phenomena is very limited at the present time. In this sense, the increased pollination efficiency in *Asclepias* is a red herring. Other consequences of the morphological changes triggered by the evolution of late-acting self-incompatibility include low multiple paternity of fruits (e.g., Broyles & Wyatt, 1990, 1995; Gold & Shore, 1995) and low variation in seed number per fruit (e.g., Wilbur, 1976; Wyatt, 1977).

Rosatti (1989: 336) summed up the confusion with respect to gynoeceal evolution in the Apocynaceae by noting that “the functional significance of the peculiar structure of the gynoeceum (usually free but apically united in most Apocynaceae and all Asclepiadaceae) has not been clearly established.” In fact, secondary apocarpy is very difficult to explain in light of the numerous advantages of syncarpy (reviewed by Endress, 1982; but see Armbruster et al., 2002). Syncarpy uses wall tissue more efficiently to enclose ovules and makes possible a wider range of fruit types than does apocarpy (Stebbins, 1974). Moreover, by allowing differentiation of a compitum, it provides for more uniform distribution of pollen tubes to ovules and maximizes pollen competition.

The evolution of an apocarpous gynoeceum without a functional compitum in some Asclepiadoideae is counter to the trend toward syncarpy found in most other angiosperm lineages. Endress (1982: 48) estimated that “only about 6% of the recent angiosperm species are apocarpous.” Indeed, many basal groups of angiosperms have evolved structures that function as an “extragynoeceal compitum,” thereby benefiting from the advantages of syncarpy while retaining the plesiomorphic state of apocarpy (Endress et al., 1983). It is therefore remarkable that the otherwise highly derived floral morphology of Apocynaceae has retrogressed by evolving carpels lacking a compitum. Kunze (1991: 250) has attempted to explain this by arguing that, because the number of pollen grains in one pollinium is limited, “an equal distribution to both ovaries would lead to many unfertilized ovules among fertilized ones.” This inefficient filling of pods should be disfavored by natural selection. This interpretation, however, is problematic. Although the pollinia of some advanced Asclepiadoideae, including some (but not all) species of *Asclepias*, do not contain enough pollen grains to fertilize all of the ovules in both ovaries (Wyatt et al., 2000), we doubt that the incredibly low pollen:ovule ratios evolved before the loss of the compitum. Instead, the numbers of pollen grains per pollinium and ovules per ovary should evolve together to ensure that each pollinium contains enough pollen grains to

fertilize all of the ovules in both ovaries. Kunze’s (1991) argument also fails to explain how selection could drive a plant to give up a trait that allows two fruits to mature from a single pollination.

PARALLEL EVOLUTION IN OTHER GROUPS OF ANGIOSPERMS

Many authors have noted the remarkable parallels between the Orchidaceae and the Apocynaceae, the only families in which pollinia have evolved. We doubt, however, that our explanation for the evolution of pollinia in milkweeds has any bearing on the parallel trend in orchids. Most orchid species are self-compatible (Gill, 1989), and we are not aware of any reports of late-acting phenomena in self-incompatible taxa. Rather than incompatibility mechanisms driving speciation, van Steenis (1969) has argued that orchid species evolve through the development of pollinator specificity. It is possible that the evolution of pollinia in orchids has been driven by selection to increase pollination efficiency. Certainly, a number of other characteristic features of orchids seem to be adaptations to overcome or compensate for low rates of pollination (e.g., many seeds per fruit and extremely long-lived flowers; van der Pijl & Dodson, 1966). As Stebbins (1974: 294) has argued, “Since orchid flowers are so specialized for cross-pollination that each flower is usually visited only once by a vector, a mechanism that facilitates the transport of a large number of pollen grains as a single unit would obviously have a great selective advantage in this family.”

It might appear that the evolutionary trends in the APSA clade that culminate in the elegant pollinaria and the complex style-head with five stigmatic regions communicating with two apocarpous ovaries are unique and without parallel. In fact, however, there are numerous instances of parallel trends in pollen delivery and receipt that may have evolved to minimize the likelihood of mixed pollen loads or to mitigate their negative consequences. Here we discuss some of these trends in groups for which there is at least some evidence for late-acting self-incompatibility, possibly with the same genetic basis and consequences as in *Asclepias*. This list is not exhaustive, and we expect that as more attention is paid to these phenomena, additional features will prove to be explicable on the basis of preventing mixed pollen loads. We challenge experts in each of these groups to reexamine morphological character evolution in light of our explanations of androeceal and gynoeceal evolution in the APSA clade.

Perhaps the most directly analogous patterns to those we have outlined occur in the legumes (Fabaceae), in which self-incompatibility is common

and widespread (Arroyo, 1981). In the two subfamilies that include more tropical and woody taxa, Mimosoideae and Caesalpinioideae, more than 60% of tested species were self-incompatible, whereas among the Faboideae the figure was less than 25%. The genetic basis of self-incompatibility in the Faboideae involves a single locus that behaves in the classical gametophytic mode (de Nettancourt, 1977). There are no definitive genetic analyses of self-incompatibility in Caesalpinioideae and Mimosoideae, but there are hints that these systems do not fit the traditional model of gametophytic self-incompatibility (Sage et al., 1994). In *Acacia retinodes* Schltdl. (Mimosoideae), Kenrick et al. (1986) showed that the site of arrest of incompatible self-pollen tubes is within the nucellus. Koptur's (1984: 1134) characterization of self-incompatibility in six species of *Inga* Mill. (Mimosoideae) revealed "that the incompatibility system is gametophytic in nature and that inhibition may occur in the ovary." Late-acting self-incompatibility is also suggested by observations of other Mimosoideae and some Caesalpinioideae in crossing studies of several species of tropical trees (Ruiz-Zapata & Arroyo, 1978; Bawa, 1979; Bernhardt et al., 1984; Gibbs et al., 1999).

In *Acacia*, pollen is regularly produced in clumps termed "polyads," in which grain numbers are typically 4, 8, 16, 32, or 64 (Kenrick & Knox, 1982; Knox & Kenrick, 1983). Kenrick and Knox (1982: 721) stated that "the possession of polyads is assumed to confer a selective advantage in reproduction, since in certain asclepiads and higher orchids the composite pollen units provide an efficient means of dispersal by insects." Again, we contend that this interpretation is probably incorrect and that, instead, polyads evolved as a means of reducing the likelihood of mixed pollen loads reaching the stigmas of plants with a wasteful late-acting self-incompatibility system. Another curious feature of *Acacia* is the modification of the stigma into a cup (Kenrick & Knox, 1981), which often is of appropriate size to receive only a single polyad. Knox and Kenrick (1983) studied pollen transfer in a natural population of *A. retinodes* and found that pollinated stigmas typically received only a single polyad. We interpret the stigma cup of *Acacia* as a mechanism, like the restricted stigmatic areas of *Asclepias*, to reduce the likelihood of mixed pollen loads. Finally, the reproductive situation in *Acacia* is similar to that of *Asclepias* in that fruit-set is remarkably low (Arroyo, 1981; Bernhardt et al., 1984). We propose that this is related to high levels of geitonogamous pollen transfer and the comparatively rare event of a solitary compatible polyad reaching a stigma.

A strikingly similar set of evolutionary trends occurs in the large, tropical genus *Inga*. Koptur (1983, 1984) has shown that these trees produce pollen in polyads of

16 to 32 grains. As in *Acacia*, there is a correspondence between grain number and ovule number, and ratios of the two fall into the range 0.95–1.74. Thus, delivery of a single polyad to a stigma provides enough pollen grains to fertilize all of the ovules within an ovary. Also, as in *Acacia*, the stigmas of *Inga* are small and, although more than one polyad will fit, the number is limited (3 to 6 according to Koptur, 1983). In all seven species studied by Koptur (1984), the majority of stigma cups (53%–73%) sampled from natural populations contained only a single polyad. It is notable too that *Inga* is characterized by very low fruit-set despite "no shortage of pollination" (Koptur, 1984: 1140). Koptur (1984) attributed this to self-polyads and incompatible cross-polyads mechanically blocking access to stigmas, rather than the more insidious effects of incompatible pollen in a late-acting system of recognition and rejection.

Other evidence for parallel evolution may be found in the Ericaceae. In several of its subfamilies (e.g., Pyroloideae, Ericoideae, Epacridoideae, Empetradoideae), individual microspores fail to separate, so that entire tetrads are transported as units by pollen vectors (Stebbins, 1974; Copenhaver, 2005). This trend reaches its apogee in the subfamily Rhododendroideae, in which "pollen tetrads often are coalesced into masses by cobwebby viscin strands; the entire anther contents may then be pulled out by the pollinator and deposited on the protruding stigma of the next flower" (Zomlefer, 1994: 81). Late-acting self-incompatibility is implicated in several species of *Rhododendron* L. (Sage et al., 1994). Williams et al. (1984) reported no difference in the growth rates of self- and cross-pollen tubes, as did Padrutt et al. (1992: 656), who further postulated "some active form of self-recognition and rejection" in *R. prinophyllum* (Small) Millais. It is therefore tempting to speculate that this wasteful, late-acting self-incompatibility has selected for increased agglutination of pollen to decrease the likelihood of mixed pollen loads.

It is also possible that late-acting self-incompatibility played a role in the evolution of secondary apocarpy in the Malvales (Sterculiaceae) and the Sapindales (Rutaceae) (Endress et al., 1983), the two families outside the Gentianales in which apocarpy occurs. Late-acting self-incompatibility has been suggested for some members of these families (Sage et al., 1994). Our lack of familiarity with these taxa makes us hesitate to suggest that secondary apocarpy has evolved in these instances under the same selection pressure as in Apocynaceae, but we hope that others will evaluate this possibility more fully.

Taken as a whole, we believe that a strong case can be made linking late-acting self-incompatibility and the

evolution of certain androecial and gynoecial characters. On the male side, these include the agglutination of pollen via retention in tetrads or polyads and via coherence due to viscin threads or exinal connections. On the female side, innovations include secondary apocarpy, stigmatic receptacles for individual pollen units, and possibly sensitive stigmas that close after a single pollination event. Plants with late-acting self-incompatibility syndromes are also characterized by low levels of fruit-set ($< 10\%$), which appear to be at least partially a consequence of the highly deleterious effects of geitonogamy and mixed pollen loads, as even the most successful adaptations are not 100% effective. Ironically, one of the reasons that late-acting self-incompatibility has proved recalcitrant to experimental genetic analysis is that plants afflicted with it have notoriously low fruit-set. In addition, researchers have been reluctant to undertake the necessary diallel crosses using long-lived woody plants. We predict that more taxa with reproductive behavior that has heretofore been enigmatic will be discovered to possess late-acting self-incompatibility. We also expect that researchers will find further examples of the sorts of adaptations we have described, which prevent mixed pollen loads or which mitigate their negative consequences.

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